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Comparison of Direct Electron Microscopy, Immune Electron Microscopy, and Rotavirus Enzyme-Linked Immunosorbent Assay for Detection of Gastroenteritis Viruses in Children

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An approximate 10% suspension in water of the first available stool sample from 411 infants and young children with acute gastroenteritis was examined by electron microscopy (EM) after 2 min of negative staining. This procedure enabled the detection of 88% of the 199 rotavirus infections, all of the 22 adenovirus infections, and 47% of the 15 ~27-nm virus infections ultimately detected by a combination of techniques, including immune electron microscopy (IEM) and rotavirus enzyme-linked immunosorbent assay (ELISA). Of the 204 infections detected by direct EM of stools, 76% were detected within 2 min of viewing, and 94% were detected within 6 min of viewing. Type 1 and type 2 rotavirus particles were visualized with approximately equal efficiency, although type 2 rotavirus infections were more common. Rectal swab preparations were clearly inferior to stool preparations for the detection of virus infection by direct EM. IEM examination was required for efficient visualization of viruses in rectal swab specimens. ELISA was the most sensitive method for the detection of rotaviruses; with this method, all infections in which rotavirus particles were visualized by EM or IEM were detected. However, 73% of the 1,834 specimens which were presumptively positive for rotavirus by conventional indirect ELISA proved to be falsely positive on the basis of EM, IEM, blocking ELISA, confirmatory ELISA, or a combination of these methods. False-positive rotavirus ELISA reactions apparently were eliminated when fecal specimens were tested in a modified confirmatory ELISA with a lower dilution of rotavirus-negative (pre-immunization) than rotavirus-positive (post-immunization) capture antibody from the same animal.

A million or more morphologically characteristic rotavirus particles are often present in 1 g of stool from a child with acute gastroenteritis (2). Electron microscopy (EM) can be used to detect the presence of these and other viruses in unconcentrated fecal samples within minutes of sample collection; thus, direct EM procedures can be particularly valuable for clinical diagnostic purposes and for following outbreaks of naturally acquired or nosocomial viral gastroenteritis. However, the relative sensitivity of direct EM as compared with other methods for detecting virus infection needs to be better established, especially now that at least two different rotavirus serotypes have been recognized (3, 6, 10) and their occurrence with respect to patient age has been shown to be different (1). This report describes the advantages, limitations, and relative sensitivities of direct EM as compared with immune electron microscopy (IEM) and rotavirus enzyme-linked immunosorbent assay (ELISA) procedures for the detection of viruses in fecal specimens of children with gastroenteritis and also describes an improved method for avoiding false-positive ELISA results.

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MATERIALS AND METHODS

Fecal samples were collected from stools (including diaper scrapings and diaper fluids) and on cotton-tipped rectal swabs. Typically, each rectal swab was swirled in, and then broken off into, a vial containing 2 ml of veal infusion broth with 0.5% bovine serum albumin. Direct EM fecal preparations were made

from a few drops of approximately 10% stool suspensions (on a vol/vol basis) in deionized water and from rectal swab specimen fluids. An equal volume of 1.5 to 2% phosphotungstic acid (brought to pH 7.1 with NaOH) was added as a negative stain. The mixtures were then dropped on Formvar-carbon-coated 400-mesh copper EM grids which were blotted dry after standing for 2 min.

IEM procedures were performed on rectal swab specimens which had been further diluted twofold in veal infusion broth containing 0.5% bovine serum albumin and on 2 to 3% stool suspensions in the same medium. Rectal swab samples were used without further clarification, whereas stool suspensions were clarified by low-speed centrifugation at 4°C for 1 h. An 0.8-ml amount of the above fluids was mixed with 0.2 ml of a 1:5 dilution of a commercial pool of immune serum globulin (human), incubated at room temperature for 1 h, and then centrifuged for 90 min at 20,000 rpm at 4°C in a Sorvall RC5 centrifuge with an SS 34 rotor (relative centrifugal force = 48,246 *g*). The resulting pellet was suspended in approximately 0.08 ml of distilled water, mixed with an equal volume of phosphotungstic acid, and dropped on electron microscopic grids. The grids were blotted dry after 2 min.

For most of the EM and IEM studies, a clock timer was started and the grids were read under code at ×36,000 or greater, usually in an RCA EMU 4C microscope. The elapsed time to the first definite recognition of a relevant virus particle was recorded. Also recorded for positive specimens was an estimate of the number of virions seen per min, using the following scale: 0 to 1+ (<1 virion); 1+ (1 virion); 2+ (5 virions), 3+ (50 virions); and 4+ (>100 virions).

Fecal specimens were also tested by the conventional (negative-specimen control) indirect ELISA procedure of Yolken et al. (7), using the same reagents or reagents very similar to those originally described. Presumptively positive specimens were confirmed by EM, IEM, blocking ELISA, (7) confirmatory ELISA (4), which utilized rotavirus-negative and rotavirus-positive capture antibody from the same animal, a modification of the latter test (see below), or a combination of these methods. In both confirmatory ELISA tests, specimens were considered positive for rotavirus if the optical density produced by reaction with a *p*-nitrophenyl phosphate substrate was above a minimum acceptable density and was at least twofold higher in wells coated with post-immunization serum than in those coated with pre-immunization serum. Most specimens found positive for rotavirus by EM, IEM, or ELISA were typed by ELISA (9), using type-specific guinea pig antisera supplied by G. Zissis (10).

RESULTS

Sensitivity of ELISA for detecting rotavirus. At Children's Hospital, 5,626 fecal preparations from infants, children, and adults were tested by conventional rotavirus ELISA. Approximately two-thirds of these specimens, including all of those from patients who were ill with diarrhea and all of those which were found positive by a confirmatory ELISA, were also tested by direct EM, IEM, or both. Rotavirus particles were visualized in 476 of these preparations, whereas rotavirus antigens were detected by ELISA in all of the samples which were found to be positive by EM or IEM and in 14 (all positive by our improved confirmatory ELISA) in which rotavirus was not visualized during repeated attempts. Several electron microscopic preparations, particularly those made from dilute rectal swab suspensions or from specimens collected late in the course of illness, were found positive only after ≥0.5-h readings following the detection of rotavirus by ELISA. Thus, ELISA has been our most sensitive method for the detection of rotaviruses in fecal specimens.

Limitations of ELISA for rapid diagnosis. Apart from the several hours needed to complete even the most rapid ELISA and the inherent inefficiency of ELISA for testing single specimens (with multiple controls) as soon as the specimen is received, misleading ELISA reactions have been a major impediment to the use of conventional ELISA as the basis for rapidly reporting rotavirus infections seen at Children's Hospital.

Among the 5,626 fecal specimens tested, 1,344 gave a positive ELISA reaction with the conventional ELISA test which could not be confirmed by visualization of rotavirus particles, blocking ELISA, confirmatory ELISA, or a combination of these methods. Thus, 73% of the 1,834 presumptively positive ELISA tests were not, in fact, positive. False-positive ELISA reactions were especially common with specimens from hospitalized young infants in a tertiary care nursery. Such false reactions have also been demonstrated at Children's Hospital with a virus-free broth culture of a protein A-producing strain of *Staphylococcus aureus*, which suggests that similar nonspecific binding of rotavirus antibody to intestinal bacteria or their products may have been the basis of other ELISA false-positive reactions which we have observed.

Improved ELISA. The frequency of false-positive ELISA reactions was greatly reduced when more than 1,000 fecal specimens were initially tested by a confirmatory ELISA in which wells on alternate rows of each Microtiter test plate (Cooke Engineering) were coated with rotavirus-negative (pre-immunization) or rotavirus-positive (post-immunization) antiserum from the same animal. False-positive reactions with the *S. aureus* bacterial culture were also eliminated by this type of testing.

After a series of confirmatory ELISA tests had been performed, it became apparent that a further modification of this ELISA was desirable,

since rotavirus-negative specimens as a group reacted more strongly with post-immunization serum than with pre-immunization serum. At times, this still resulted in weak false-positive reactions. Nonspecific binding of bacterial components to immunoglobulin, which would be present in greater quantity in post-immunization serum than in pre-immunization serum from the same animal, presumably was the basis for at least some of these false-positive reactions.

A series of rotavirus-negative specimens was then tested by ELISA to determine endpoint spectrophotometric readings produced with varying dilutions of pre-immunization capture antibody, as compared with the standard (1:100,000) dilution of post-immunization capture antibody. A 1:25,000 dilution of pre-immunization antibody produced the most closely equivalent mean readings and was adopted for routine use in all confirmatory ELISA tests and in those tests in which a reportable ELISA result was needed as rapidly as possible. Subsequently, in more than 400 consecutive tests with our modified ELISA, very satisfactory ELISA sensitivity has been maintained, and false positive results have apparently not been encountered.

Some question obviously must remain where any ELISA-positive specimen remains negative by EM, but these now rare instances typically relate to dilute specimens or those taken late in the course of illness when the insensitivity of electron microscopy is much more likely to be the key factor in our failure to detect virus particles.

Sensitivity of direct EM compared with ELISA. Early in the course of these studies, we found that at least some enteric virus infections could be demonstrated by direct EM after minimal specimen preparation. Accordingly, the first available stool sample from each of 411 gastroenteritis inpatients was tested for rotavirus by both direct EM and confirmed ELISA (Table 1). In these stool preparations, 87.9% of all of the rotavirus infections which were found by ELISA were detected by direct EM, including 90% of the rotavirus type 1 infections and 87% of the rotavirus type 2 infections demonstrated during the period when typing reagents were available.

Insensitivity of direct EM for rectal swab specimens. The first available rectal swab specimen from each of 105 ELISA-positive gastroenteritis inpatients and outpatients was similarly examined (Table 1). Rotavirus particles were seen in specimens from only 22 (21%) of these subjects, including 12 of 68 inpatients and 10 of 37 outpatients. Thus, the great majority of rotavirus infections in these patients would have been missed, had reliance been placed exclusively on direct EM of their rectal swab specimens.

Sensitivity of direct EM compared with IEM. EM and IEM techniques were used in parallel to detect a variety of viruses in stool samples from 362 patients and in rectal swab specimens from 123 patients (Table 2). Of 192 viruses demonstrated among inpatients' stool samples, 84.4% were demonstrated by direct EM as compared with 96.4% by IEM. In marked contrast, IEM demonstrated four times as many viruses in rectal swab preparations than were demonstrated by direct EM (Table 2).

In spite of the limitations of the direct EM technique, its rapidity and relative efficiency for testing stool samples lend it considerable value as a screening test, particularly for gastroenteritis patients with stools during periods when rotaviruses are prevalent. In fact, at Children's Hospital, the initial detection of relevant enteric viral infection in such patients is now typically made by direct EM.

Virus detection related to EM reading time. The EM reading times required to detect rotaviruses by direct EM of stools and IEM of stool samples and rectal swab specimens are shown in Table 3. Although the individuals represented in Table 3 were not necessarily tested or positive by all of the study methods, more than two-thirds of those found positive by each method were detected within the first 3 min of reading. Clearly, there was a diminishing return as reading times increased. Also, with each study method, quite similar cumulative percentages of both type 1 and type 2 rotaviruses were detected at the different time intervals.

Comparable data for adenoviruses and ~27-nm viruses are shown in Table 4; the same specimens were tested by direct EM and IEM. Most of the viruses included in Table 4 were found within 10 min of viewing. However, it should be cautioned that some additional infections, particularly by ~27-nm viruses, might have been detected if sensitive serological tests for their presence had been available or if extremely long EM reading times had been employed.

The findings (Tables 3 and 4) have led us to conclude that the most practical balance between the minimum time of electron microscopic observation and the maximum detection of viruses in fecal preparations from acutely ill patients is achieved by reading IEM preparations for no more than 10 min. For rapid diagnosis of infection where speed is of the essence, we now routinely read direct EM preparations of diarrhea stool samples for no more than 6 min.

TABLE 1. *Comparison of direct EM and ELISA for the detection of rotaviruses in the first available stool or rectal swab specimen from children with gastroenteritis*

Rotavirus serotype	Study period	Inpatient stool samples[a]			Inpatient and outpatient rectal swab specimens[b]		
		No. positive by:		EM positive/ ELISA positive (%)	No. positive by:		EM positive/ ELISA positive (%)
		EM	ELISA		EM	ELISA	
Type 1	January 75–June 78	45	50	90.0	5	27	18.5
Type 2	January 75–June 78	94	108	87.0	17	71	23.9
Total (including untyped)	January 75–April 79	175	199	87.9	22	105	21.0

[a] 318 patients were tested through June 1978; 411 were tested through April 1979.
[b] 114 patients were tested through June 1978; 123 were tested through April 1979.

TABLE 2. *Comparison of direct EM and IEM methods for the detection of viruses in the first available stool sample or rectal swab specimen from children with gastroenteritis*[a]

Virus type	Stool samples[b]			Rectal swab samples[c]		
	Total no. of viruses by EM or IEM	No. (%) of viruses by:		Total no. of viruses by EM or IEM	No. (%) of viruses by:	
		Direct EM	IEM		Direct EM	IEM
Rotavirus	30	25 (83.3)	29 (96.7)	26	5 (19.2)	26 (100)
Type 1						
Type 2	85	73 (85.9)	82 (96.5)	65[d]	17 (26.2)	65 (100)
Total (including untyped)	155	133 (85.8)	149 (96.1)	98[d]	22 (22.4)	98 (100)
Adenovirus	22	22 (100)	22 (100)	10	4 (40.0)	10 (100)
~27-nm Virus	15	7 (46.7)	14 (93.3)	2	1 (50.0)	2 (100)
Total viruses	192[e]	162 (84.4)	185[e] (96.4)	110[d]	27 (24.5)	110 (100)

[a] The study period was from January 1975 through April 1979.
[b] 362 inpatients were tested.
[c] Of 123 samples tested, 77 were from inpatients and 46 were from outpatients.
[d] One additional patient sample was found positive by ELISA.
[e] Three stools had rotavirus type 1 plus ~27-nm virus; three stools had rotavirus type 2 plus ~27-nm virus.

Centrifuged preparations without immunoglobulin. A limited attempt was also made to determine whether antibody was required for the efficient demonstration of virus in our IEM system. A total of 6 stool and 20 rectal swab preparations which were negative for rotavirus by direct EM but positive by IEM were centrifuged in a Sorvall centrifuge as if for IEM, but no immunoglobulin was added to aggregate virus. Twenty-four (92%) of these centrifuged specimens were found positive for virus, all within EM reading periods of 8 min or less. Thus, Sorvall centrifugation alone would appear to permit relatively efficient visualization of rotavirus particles in weakly positive specimens.

Other findings relating to virus positivity. Roughly 10 virions were seen per min of viewing of the typical virus-positive EM or IEM stool preparation made early in the course of patient hospitalization for acute gastroenteritis. Among virus-positive inpatients, this mean value was not found to change significantly with respect to either patient age or identified virus type or group. The number and proportion of seriously ill patients who were virus positive did, however, tend to decline with increasing age after 12 to 15 months of life. During more than 6 years of study, few fecal viruses were demonstrable by EM, IEM, or ELISA in the months of June through October. In contrast, 62% of 511

TABLE 3. *Detection of rotaviruses in the first available EM-positive fecal specimen from inpatients with gastroenteritis in relation to time of electron microscopic observation*[a]

Elapsed time (min)	Cumulative % found positive for rotaviruses by:								
	Direct EM of stool samples[b]			IEM of stool samples[c]			IEM of rectal swab specimens[d]		
	Type 1	Type 2	All rotaviruses (including untyped)	Type 1	Type 2	All rotaviruses (including untyped)	Type 1	Type 2	All rotaviruses (including untyped)
0.5	51	40	46	31	32	34	31	24	23
1	62	57	59	47	41	45	38	34	31
2	76	76	75	59	59	62	50	61	55
3	87	83	83	69	72	73	75	80	77
4	91	86	86	78	78	80	75	85	80
5	93	93	91	88	85	87	81	93	86
6	96	95	94	88	86	88	94	95	92
8	96	97	97	91	92	94	100	98	98
10	98	98	98	97	99	99	100	100	100
>10	100	100	100	100	100	100	100	100	100

[a] The study period was from January 1975 through April 1979.
[b] The total numbers of viruses were: 45, type 1; 94, type 2; and 175, all rotaviruses (including untyped).
[c] The total numbers of viruses were: 32, type 1; 87, type 2; and 157, all rotaviruses (including untyped).
[d] The total numbers of viruses were: 16, type 1; 41, type 2; and 64, all rotaviruses (including untyped).

TABLE 4. *Detection of adenoviruses and ~27-nm viruses in the first available EM-positive stool specimen from inpatients with gastroenteritis in relation to time of electron microscopic observation*[a]

Elapsed time (min)	Cumulative % found positive by:			
	Direct EM		IEM	
	~27-nm Virus	Adenovirus	~27-nm Virus	Adenovirus
0.5	43	64	36	14
1	57	73	36	18
2	71	86	50	64
3	86	91	71	68
4	100	91	79	77
5	100	91	86	82
6	100	91	93	86
8	100	95	93	95
10	100	100	93	95
>10	100	100	100	100

[a] The study period was from January 1975 through April 1979. The total numbers of ~27-nm virus and adenovirus infections detected by EM were 7 and 22, respectively; by IEM, the total numbers of ~27-nm virus and adenovirus infections detected were 14 and 22, respectively. Note that seven additional ~27-nm virus infections were detected by IEM.

hospitalized patients with diarrhea were virus positive by EM or IEM in the months of December through March.

DISCUSSION

Direct EM, IEM, and ELISA are each uniquely useful in the study of pediatric gastroenteritis. Direct EM, though it is the least sensitive technique, affords by far the most rapid method for detecting fecal viruses and has proven to be an especially useful diagnostic and teaching tool when used to test the first available stool sample from acutely ill patients with diarrhea during periods of rotavirus activity. A large majority of all viruses found in stool samples from our acutely ill gastroenteritis inpatients is now first detected by this method, and results are typically available within minutes to a few hours of the arrival of the specimen at the laboratory.

ELISA procedures are clearly the most sensitive of our methods for detecting rotaviruses, have been useful for typing these agents, and are ideal for screening large numbers of fecal specimens in a single test. The ELISA technique can be used where an electron microscope is not available and may ultimately be the most practical method for many diagnostic laboratories. However, ELISA kits for detecting rotaviruses are just beginning to appear commercially, and ELISA reagents for detecting various other intestinal viruses are not yet generally available. Also, false-positive ELISA reactions have occurred with disturbing frequency in our conventional (negative-specimen control) ELISA procedures. A modified confirmatory ELISA procedure effectively eliminated the problem of ELISA false-positive results, including those produced by a virus-free culture of protein A-producing *S. aureus*. It should also be noted that one of us (R.H.Y.) has used *N*-acetyl cysteine and other treatments to reduce nonspecific rotavirus ELISA activity in stool samples (8). These treatments were not used, and apparently

were not needed, in the present study.

The IEM technique is presently our best single method for detecting all of the known gastroenteritis viruses, especially in rectal swab specimens, but with most stool specimens, IEM takes hours longer than direct EM to provide the same diagnostic result. Neither IEM nor ELISA procedures lend themselves well to testing a single sample late in the laboratory day, a situation for which direct EM is ideally suited.

Our rectal swab preparations were strikingly inferior to stool preparations for the direct EM demonstration of virus infection. This finding has particularly important implications for the detection of gastroenteritis viruses in pediatric outpatients, from whom a stool sample may not be obtainable during a brief office visit. IEM and rotavirus ELISA were needed for the efficient detection of gastroenteritis viruses in rectal swab preparations. With this in mind, other laboratories attempting direct EM diagnosis may find it worthwhile to experiment with less dilute rectal swab preparations than we have used.

Those who use EM for the detection of fecal viruses must consider how much time and effort should be applied to a specimen before it is considered negative for recognizable pathogens. Obviously, if one does not read specimen grids long enough, a sizable fraction of the detectable viruses may not be seen. On the other hand, EM time is expensive and, particularly when rotaviruses are not epidemic, the bulk of EM reading time tends to be spent reading grids which cannot provide a diagnosis. On the basis of findings presented in the paper, we have concluded that the best use of EM time consistent with the efficient diagnosis of pediatric viral enteritis is achieved by routinely reading direct EM preparations for no more than 6 min and IEM preparations for no more than 10 min before considering them negative for virus. These times are especially relevant if the specimens are collected within the first three days of the onset of an acute gastroenteritis, while the patient still has considerable diarrhea.

A rather subtle difference between EM and IEM test results (partially evident in Tables 3 and 4) should also be noted. Virus particles tended to be visualized after a somewhat longer reading period in IEM preparations than in direct EM preparations of the same specimen. At least three factors probably are responsible: in the IEM preparations some viruses were more difficult to recognize definitively under a coating of antibody or other serum proteins; since virus in the IEM preparations often was present in relatively isolated clumps, a greater area of the IEM grid tended to be devoid of any virus; and finally, where a low-speed clarification step was used to remove bacteria, some large aggregates of virus may also have been removed (5).

In this study, type 1 and 2 rotavirus particles were visualized with approximately equal rapidity and efficiency, and all rotavirus infections were apparently detected by ELISA. The more common finding of type 2 rotaviruses than type 1 rotaviruses in our total group of study patients (1) thus indicates real differences in the epidemiology of these viruses rather than mere differences in the sensitivity of our virus detection methods.

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LITERATURE CITED

1. **Brandt, C. D., H. W. Kim, R. H. Yolken, A. Z. Kapikian, J. O. Arrobio, W. J. Rodriguez, R. G. Wyatt, R. M. Chanock, and R. H. Parrott.** 1979. Comparative epidemiology of two rotavirus serotypes and other viral agents associated with pediatric gastroenteritis. Am. J. Epidemiol. **110**:243–254.
2. **Davidson, G. P., R. F. Bishop, R. R. W. Townley, I. H. Holmes, and B. J. Ruck.** 1975. Importance of a new virus in acute sporadic enteritis in children. Lancet **i**:242–246.
3. **Flewett, T. H., M. E. Thouless, J. N. Pilfold, A. S. Bryden, and J. A. N. Candeias.** 1978. More serotypes of human rotavirus. Lancet **ii**:632.
4. **Kapikian, A. Z., R. H. Yolken, H. B. Greenberg, R. G. Wyatt, A. R. Kalica, R. M. Chanock, and H. W. Kim.** 1979. Gastroenteritis viruses, p. 964. *In* E. H. Lennette and N. J. Schmidt (ed.), Diagnostic procedures for viral, rickettsial and chlamydial infections, 5th ed. American Public Health Association, Washington, D.C.
5. **Narang, H. K., and A. A. Codd.** 1980. Preponderance of rotavirus in clumped form in patients with acute gastroenteritis. Lancet **i**:1192–1193.
6. **Ridriguez, W. J., H. W. Kim, C. D. Brandt, R. H. Yolken, J. O. Arrobio, A. Z. Kapikian, R. M. Chanock, and R. H. Parrott.** 1978. Sequential enteric illness associated with different rotavirus serotypes. Lancet **ii**: 37.
7. **Yolken, R. H., H. W. Kim, T. Clem, R. G. Wyatt, A. R. Kalica, R. M. Chanock, and A. Z. Kapikian.** 1977. Enzyme-linked immunosorbent assay (ELISA) for detection of human reovirus-like agent of infantile gastroenteritis. Lancet **ii**:263–267.
8. **Yolken, R. H., and P. J. Stopa.** 1979. Analysis of nonspecific reactions in enzyme-linked immunosorbent assay testing for human rotavirus. J. Clin. Microbiol. **10**: 703–707.
9. **Yolken, R. H., R. G. Wyatt, G. Zissis, C. D. Brandt, W. J. Rodriguez, H. W. Kim, R. H. Parrott, J. J. Urrutia, L. Mata, H. B. Greenberg, A. Z. Kapikian, and R. M. Chanock.** 1978. Epidemiology of human rotavirus types 1 and 2 as studied by enzyme-linked immunosorbent assay. N. Engl. J. Med. **299**:1156–1161.
10. **Zissis, G., and J. P. Lambert.** 1978. Different serotypes of human rotaviruses. Lancet **i**:38–39.